Volatile Male-specific Products of Fruitspotting Bugs (Heteroptera : Coreidae)

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Introduction

Fruitspotting bugs are major pests of tree fruit and nut crops grown along the coastal strip from northern NSW to Torres Strait. Their importance continues to increase as the variety of susceptible host crops and the area devoted to their culture increases. The fruitspotting bug *Amblypelta nitida* Stal and the banana spotting bug *A. lutescens lutescens* (Distant) (Hemiptera : Coreidae) attack virtually every tropical and subtropical fruit and nut crop grown commercially in Queensland, as well as curiosities such as feijoa, pomegranate, acerola and grewia.

The bugs are primarily pests of green fruit, and can cause total crop loss if they are not controlled with pesticide applications. Feeding on fruit may lead to fruit abscission (lychees, longans, macadamias, mangoes) or mature fruit may be rendered cosmetically unsuitable for market (avocados, guavas, custard apples, passionfruit). In addition, the lesions caused by their feeding activity may provide points of entry for microbial infections (anthracnose on avocados), although such fruit would generally be rejected regardless of subsequent infection.

Historically, *A. nitida* has been the predominant species affecting commercial fruits in the coastal region just north of Brisbane. Lately, however, *A. lutescens* has consolidated its previously minor presence in this region, possibly due to the combined effects of the increase in the availability of alternative ornamental hosts associated with the residential population explosion, and the expanding area of susceptible crops. Many new households, particularly those on the numerous residential acreages, also have backyard fruit trees (*e.g.*, avocado, lychee, custard apple, *etc.*) which are generally not sprayed. Whatever the reason, *A. lutescens* is probably of equal pest status with *A. nitida* in the south Queensland coastal region. This means "double trouble" for growers of avocado, lychee, guava, macadamia and mango in the area. It also means increased trouble for growers of papaws and custard apples which are attacked only by *A. lutescens*.

Because of the importance of the pests, and the complications caused to development of IPM systems by the need to spray regularly, an alternative control tactic, or at least a method of accurately monitoring *Amblypelta* infestations, is highly desirable. Such a development might make bug control more efficient, and perhaps reduce the total number of sprays applied. Biological control options appear to be almost nil, so research into the potential for the use of pheromones, at least for monitoring purposes, was initiated.

Methods

Cultures of both species of *Amblypelta* were continuously augmented with field-collected specimens. Twenty-five to 100 nymphs were reared in individual cages in the insectary on a diet of green beans. Newly ecdysed adults were removed from these cages daily to obtain groups of virgin males and females for dissection and aeration experiments.

Males and females were dissected under a stereomicroscope to determine if exocrine glands, which might produce a pheromone, were present. Males were examined critically for the presence of the ventral abdominal gland (VAG) described by Thouvenin (1965) as being present in many coreid species.

Up to 50 virgin bugs of either sex were placed in a 50 mm diameter glass tube and air was drawn through by suction via an activated charcoal filter. The outgoing airstream (flowing at approximately 60 ml per min.) passed through a luer-lock filter packed with activated charcoal. A fine wire gauze platform was provided for the bugs to rest upon, and distilled water was provided via a vial and wick. Each aeration was run for 20–28 hours.

Extraction of volatiles collected on the charcoal in the luer-lock filter was carried out using approximately 300 ul of hexane or dichloro-methane. These extracts were analysed on a gas chromatograph (GC) or a gas chromatograph-mass spectrometer (GC-MS). Compounds were identified by GC-MS and verified by co-chromatography of synthetic standards. The allomones of adults and nymphs of both species were also identified so that contamination from the scent glands could be recognised, should it occur.

Identified volatiles were tested for attractiveness in the laboratory in a Y-tube olfactometer and in the field by attaching a cigarette filter containing 50 ul of the test blend to a plastic tub or ball coated with Bird-Off®.

Results and Discussion

Male-specific volatile blends were isolated from both spotting bug species, first for *A. lutescens* (Figure 1), and then as sufficient numbers became available, for *A. nitida* (Figure 2). Six volatiles were repeatedly detected in *A. lutescens* male extracts; these were never detected in extracts from females. Four of these components have been identified, with co-chromatography of synthetic standards, as beta-ocimene, linalool, alpha-farnesene and (E)-nerolidol. Linalool and (E)-nerolidol are optically active compounds. *A. lutescens* produces only the enantiomer (-)-3R-nerolidol, while the chirality of linalool remains to be determined. Four geometrical isomers are present in alpha-farnesene, and the *A. lutescens* farnesene has been tentatively identified as the (E,E) isomer. Components with retention times (RT) of 8.65 and 13.40 minutes remain unidentified. However, a sample analysed by the USDA Beltsville Laboratory by chemical ionization-MS indicated that the RT = 8.65 component has a molecular formula of $C_{10}H_{16}O$, with the oxygen moiety being non-alcoholic. The RT = 13.40 component has a molecular weight of 220, and apparently lacks an -OH moiety.

While the $C_{10}H_{16}O$ compound (RT = 8.65) is the least abundant of the six male-specific *A. lutescens* volatiles, it is clearly the major male-specific compound in *A. nitida* (Figure 3a). Less abundant components occurring in extracts from *A. nitida* males at RT = 8.55 and RT = 9.40 appear to be chemically closely related to the RT = 8.65 component. In addition, a trace of the RT=13.40 compound (verified by GC-MS) is present in extracts from *A. nitida* males, but not even a trace of the male *A. lutescens* major component (RT = 12.93; (E)-nerolidol) has been detected from *A. nitida* males. Thus, these male-specific volatile blends from *Amblypelta* are also highly species specific, a pattern that suggests that these blends are indeed sex pheromones.

A blend of the four identified compounds from *A. lutescens* was prepared to test in the field. The areas from the GC integrator for these four components were used to calculate percentages for each in the blend. The percentages of the two chiral compounds linalool and (E)-nerolidol were doubled initially on the assumption that the insects produce one or another enantiomer. Each percentage was then multiplied by the reciprocal of the purity (as determined by GC) of the desired isomer in each synthetic standard. The crude synthesised blend was used to bait sticky traps which were hung, along with unbaited control traps, in five different trees known to be hosts of *Amblypelta* spp. at Maroochy Research Station. Over the period 12 March to 18 March 1991, no bugs were caught in the traps, but several adults were observed within about 30 cm of various traps. Trapping was repeated during the summer of 1992 using the four-component blend and the pure (-)-trans-nerolidol [(-)-3R-nerolidol was not available]. Again no bugs were caught, even though on several occasions bugs were common on the trap trees. Olfactometer tests in the laboratory also failed to attract virgin females to test baits.

While six components have been shown by repeated aerations to be produced by male *A. lutescens*, nerolidol is by far the most abundant, and in numerous aerations

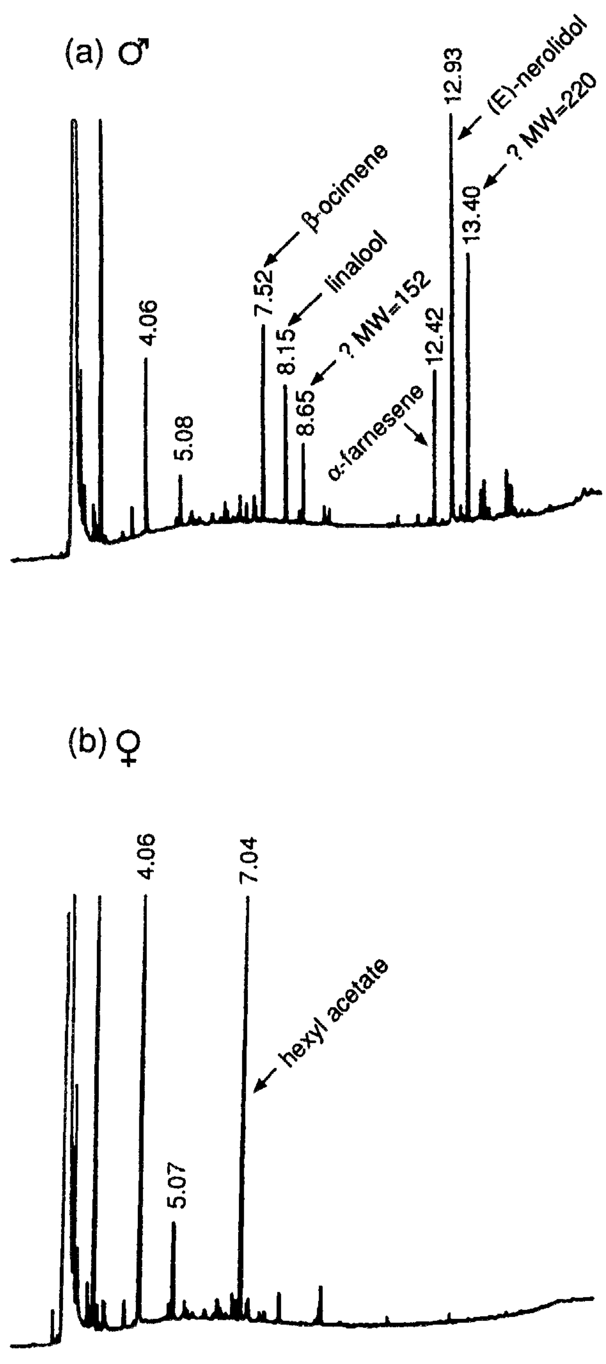

Figure 1 GC traces of *A. lutescens* males (a) and females (b)

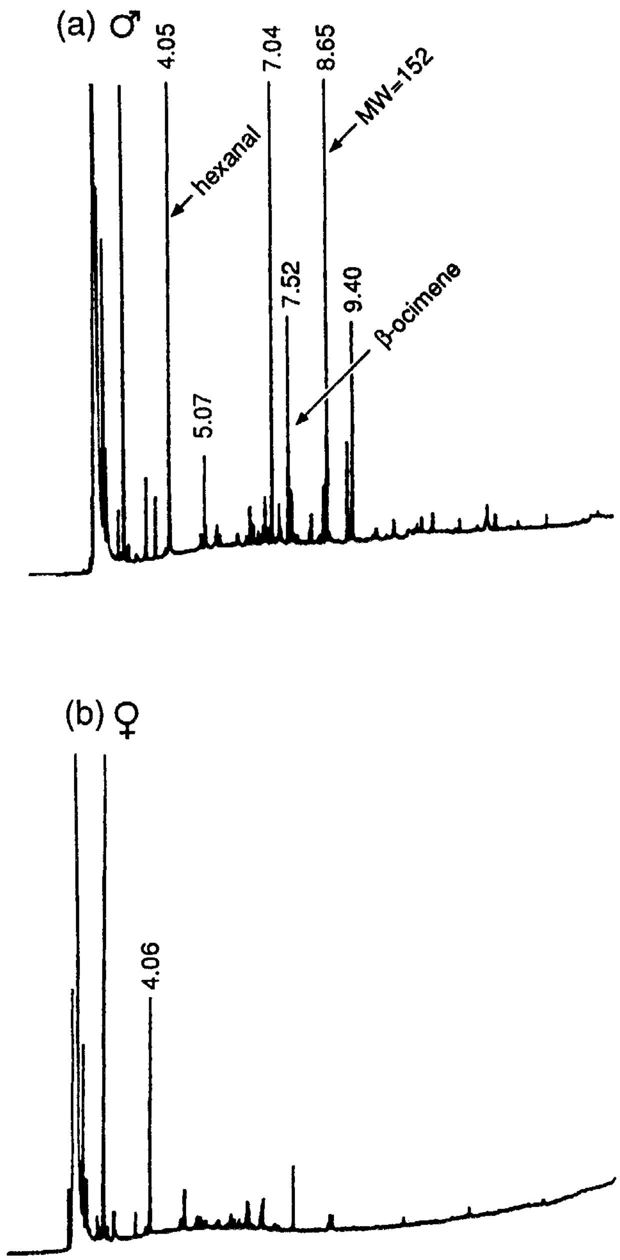

Figure 2 GC traces of *A. nitida* males (a) and females (b)

was present when the others were barely detectable or were absent. This has led us to believe that (-)-3R-nerolidol, by itself, could be an attractant. If this is so then the lack of attraction of both the baits tested may have been caused by an inhibitory effect of the (+)-3S-nerolidol present in the mixture. So far we have been unable to source pure (-)-3R-linalool from which (-)-3R-nerolidol could be synthesised.

The RT = 8.65 compound (MW = 152) which occurs in the *A. lutescens* blend and which is the major component of the *A. nitida* blend, also occurred alone in several aerations. This suggests the possibility that this compound alone may be the major attractant for *A. nitida*. Positive identification of the substance will probably require large amounts which could take some time to accumulate, given the relatively poor performance of the *A. nitida* laboratory cultures compared to those of *A. lutescens*.

Our conclusions are then that the males of both *A. lutescens* and *A. nitida* produce chemical blends which are not produced by the females, and that these blends are probably sex pheromones. True bugs are "chemical factories", producing a range of defensive chemicals as well as possible pheromones. This has often confounded the task of isolating sex pheromones (Aldrich, 1988). Indeed, hemipteran pheromone research has progressed slowly compared to that for Lepidoptera and some other insect orders. Although it is possible that pheromones are not as prevalent in Hemiptera, the truth may be that true bugs have not been as extensively studied as, for example, the Tortricidae. Also, some researchers have been looking for pheromones in the wrong exocrine gland (Aldrich, 1985).

For both *Amblypelta* species we know the components of the defensive secretions produced by the metathoracic glands. These secretions are made up of aldehydes, alcohols and esters. The volatiles captured from males are sesquiterpenes which have a pleasant, sweet smell. Identification of the remaining two unknown compounds from *A. lutescens* will allow us to have more confidence in the results of attractancy tests. Since one of these unknowns is the major component in the *A. nitida* blend, we would also make major progress with that species.

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